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Application No. 09/657289	Prepared by	BRS	Tracking Number
Examiner-GAU Slobodyansky 1652	Date	1-27-04	Week Date
	No. of queries	1	AP

JACKET			
a. Serial No.	f. Foreign Priority	k. Print Claim(s)	p. PTO-1449
b. Applicant(s)	g. Disclaimer	l. Print Fig.	q. PTOL-85b
c. ~~Continuing Data~~	h. Microfiche Appendix	m. Searched Column	r. Abstract
d. PCT	i. Title	n. PTO-270/328	s. Sheets/Figs
e. Domestic Priority	j. Claims Allowed	o. PTO-892	t. Other

SPECIFICATION

a. Page Missing
b. Text Continuity
c. Holes through Data
d. Other Missing Text
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g. Brief Description
h. Sequence Listing
i. Appendix
j. Amendments
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CLAIMS

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Thanks

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RESPONSE Provisional data inserted on p. 1.

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LUCIFERASE EXPRESSION CASSETTES AND METHODS OF USE

This application claims the benefit of provisional application No. 60/152904, filed Sept. 8, 1999.

TECHNICAL FIELD

The present invention relates to luciferase expression vectors, methods of making same and methods of use thereof.

BACKGROUND OF THE INVENTION

Bioluminescent bacteria are widely found in both marine and terrestrial environments. Interestingly, all identified species of naturally occurring marine and terrestrial bioluminescent bacteria are Gram-negative. To date, at least eleven species in four Gram-negative genera have been described: *Vibrio*, *Photobacterium*, *Shewanella* (*Altermonas*) and *Photorhabdus* (*Xenorhabdus*). In all these species, the five genes responsible for bioluminescence are clustered in the *lux* operon (*luxCDABE*).

The bioluminescence (emitted blue-green light having a wavelength of about 490 nm) is thought to result from a luciferase-catalyzed oxidation of reduced flavin mononucleotide ($FMNH_2$) and a long-chain fatty aldehyde. The luciferase enzyme is encoded by two subunits (*luxAB*), whereas the fatty acid reductase polypeptides responsible for the biosynthesis of the aldehyde substrate for the luminescent reaction are encoded by the three genes *luxCDE*. The genes encoding luciferase and the fatty acid reductase polypeptides have been cloned from the *lux* operons of *Vibrio*, *Photobacterium* and *Photorhabdus* and sequenced. In each case, the *luxCDE* genes flank the *luxAB* genes, with transcription in the order *luxCDABE*. Although a number of additional *lux* genes have been identified in each of these three bacteria, only *luxA-E* are essential for the biosynthesis of light (reviewed by Meighen, E., (1993,*The FASEB Journal* 7:1016-1022 and Ulitzur, S., (1997), *J. Biolumin Chemilumin* **12**:179-192).

Methods described in U.S. Patent 5,650,135, make possible the detection of bioluminescent bacteria in a living animal without dissecting or otherwise opening the animal up ("*in vivo* monitoring") – the light is detected through muscle, skin, fur & other traditionally "opaque" tissues using a highly sensitive camera. In this context and others, it would therefore be desirable to confer bioluminescence properties on a bacterium of one's choice, so that the bacterium could be followed with *in vivo* monitoring in various models of infection. In particular, it would be desirable to confer such bioluminescence properties on Gram-positive